



Atty. Docket No. 1510-1011
PATENTS

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Antoni BANAS et al.

Confirmation No. 4182

Serial No. 09/709,457

GROUP 1638

Filed November 13, 2000

Examiner R. Kallis

USE OF CLASS ENZYMES AND THEIR
ENCODING GENES TO INCREASE THE
OIL CONTENT IN TRANSGENIC
ORGANISMS

DECLARATION UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Oliver OSWALD, hereby declare as follows:

I am a scientist at BASF Plant Science GmbH in Germany. My relevant background and experience are set forth on the attached Curriculum Vitae. I make this declaration in support that one of ordinary skill in the art would be able to make and use the claimed invention.

As evidence of this assertion, enclosed are results of a study analyzing the triacylglycerol content of *Brassica napus* seeds that over express the ARE1 gene of *Saccharomyces cerevisiae*. The study shows that *Brassica napus* plants that over express the yeast ARE1 gene exhibit a significant increase in oil content. These studies have been conducted in view of the present application and techniques known to a person working in the area of plant genetics.

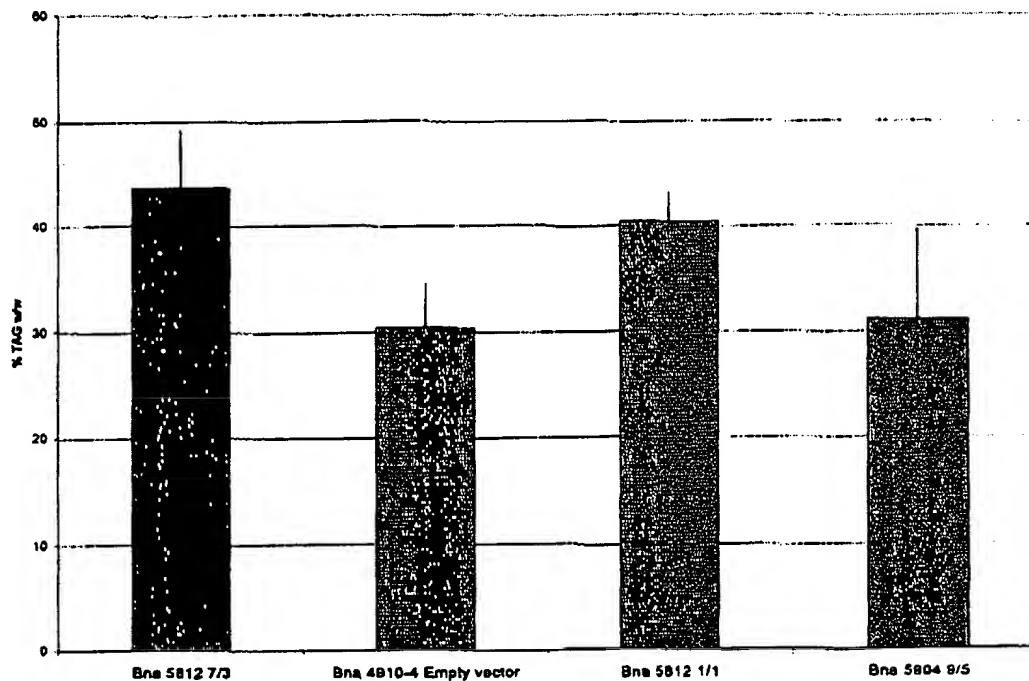
The study may be characterized as follows:

Brassica napus lines expressing the yeast ARE1 gene from a napin promotor were generated by *Agrobacterium* transformation. The oil content in T1 seeds from transformed lines with confirmed yeast ARE1 expression were analyzed and compared to seed oil content in control plants transformed with an empty vector.

The extraction of lipids from the seeds was performed according to Bligh & Dyer (1959) Can J Biochem Physiol 37:911. The seed material was broken down by the use of a ball mill (MM300 of company Retsch (Haan)). The solvent used was Chloroform/Methanol (2:1; containing Mono-C17-glycerol from Sigma as internal standard). After addition of potassium phosphate buffer having a pH of 7.5, phase separation was initiated. As to the organic phase, an aliquot was taken, diluted with chloroform, and spotted onto the capillary Chromarods SIII of the company Iatroscan (SKS, Bechenheim). A two-step separation was performed by thin layer chromatography in 6:2:2 Chloroform: Methanol: Toluol followed by 7:3 n-Hexane:Diethylether as a solvent mix. The samples were measured using a Iatroscan MK-5 (SKS, Bechenheim) according to Fraser & Taggart, 1988 J. Chromatogr. 439:404. Quantification was performed using the internal standard and calibration curves with Tri-C17-glycerin (Sigma) and the software ChromStar (SKS, Beichenheim).

Brassica napus plants over expressing the yeast ARE1 gene (identified as line Bna 5812-7 show a significant

increase in oil content as compared to plants that do not (identified as Bna 4910-4 empty vector control) ($p=0,026299756$). The results are shown in the figure below. The results are based on the analysis of 10 individual plants per line with 3 separate extractions per plant.



It is believed that the results of the present study demonstrate that the present disclosure is enabling for a method for increasing the oil content of an organism as claimed.

The undersigned declares further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or

both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 27.5.2003

Oliver Oswald
Dr. Oliver OSWALD

CURRICULUM VITAE

I, Oliver Oswald, holding a PhD in plant biochemistry, a citizen of the Federal Republic of Germany and residing at Schwarzwaldstr.1, D-68163 Mannheim, Germany, declare as follows:

I am a fully trained biologist, having studied biology at the University of Mainz from 1993 to 1995 and the University of Glasgow from 1995 to 1997;

I obtained my doctor's degree from the University of Glasgow in 2001;

Since 2001, when I joined BASF Plant Science GmbH, Germany, I have been working in the field of plant molecular biology and plant genetic engineering;

I am not one of the inventors of the invention disclosed and claimed in Application Ser. No. 09/709,457 but as I am working in the same research field I am familiar with the contents thereof;

I further declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.



Dr. Oliver Oswald

Signed at Mannheim, Germany,

this 27th day of May, 2003